

CYTOGENETIC STUDIES OF COMMON BEAN (Phaseolus vulgaris) AND
THE SCARLET RUNNER BEAN (Phaseolus coccineus)

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1979

TO

Elizabeth, my wife

Dr. Tien-Ho, my father

Dr. Su-Su, my mother

ACKNOWLEDGMENTS

The author expresses his sincere gratitude to Dr. Mark J. Bassett, the chairman of his graduate committee, for his unusual ability to "Kai-chiau" (open the doors of thinking), and his patience as an educator. The author also expresses his thanks to Dr. Kenneth H. Quesenberry and his group of cytogenetic co-workers for the orientation and facilities generously provided. Thanks are extended to the faculty members, staff and all the graduate students of the Vegetable Crops Department for the good times experienced during the author's pursuit of knowledge at the University of Florida.

Special thanks are extended to the Brazilian Ministry of Education and the Escola Superior de Agricultura de Lavras for financial support during the author's training.

Finally, thanks are extended to Elizabeth, his wife, for her patience during his ordeal.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iii
ABSTRACT	vi
INTRODUCTION	1
PART I	
MEIOSIS IN COMMON BEAN (<u>Phaseolus vulgaris</u>)	
INTRODUCTION	4
LITERATURE REVIEW	6
MATERIALS AND METHODS	9
RESULTS AND DISCUSSION	12
Slide Preparation	12
Meiotic Sequence	14
SUMMARY	28
PART II	
CYTOGENETIC ANALYSIS OF INTERSPECIFIC HYBRIDS BETWEEN COMMON BEAN (<u>Phaseolus vulgaris</u>) AND THE SCARLET RUNNER BEAN (<u>Phaseolus coccineus</u>)	
INTRODUCTION	30
LITERATURE REVIEW	32
MATERIALS AND METHODS	39
RESULTS AND DISCUSSION	41
Pollen Abortion Analysis	41
Gross Chromosome Homology Between the Genomes	45
Anaphase I Analysis	46
Anaphase II Analysis	51
Unequal Distribution of Chromosomes at Anaphase I	54
Normal Pollen Abortion	55

TABLE OF CONTENTS (Continued)

	Page
PART II (Continued)	
RESULTS AND DISCUSSION (Continued)	
F ₂ Seed Development	55
Aneuploid Progeny in Interspecific F ₂	56
CONCLUSIONS	57

PART III

CHROMOSOME IDENTIFICATION AT THE DIPLOTENE STAGE OF
MEIOSIS IN SCARLET RUNNER BEAN (Phaseolus coccineus)

INTRODUCTION	59
LITERATURE REVIEW	62
MATERIALS AND METHODS	66
RESULTS AND DISCUSSION	68
CONCLUSION	85
SUMMARY	86
REFERENCES	87
BIOGRAPHICAL SKETCH	90

Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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August 1979

Chairman: Mark J. Bassett
Major Department: Vegetable Crops

Sixty photomicrographs of the meiotic sequence of common bean (Phaseolus vulgaris) were presented to discuss the peculiarities of meiotic configurations in bean meiosis. The advantages and limitations of cytogenetic research in bean were discussed and compared with the meiotic configurations observed in other plants, especially tomato and maize.

A detailed quantitative analysis of chromosomal behavior during the meiotic process in the pollen mother cells (PMCs) of interspecific hybrids from P. vulgaris x P. coccineus crosses was conducted to find the cause of the high degree (59-79%) of pollen sterility in F_1 plants. Contrary to the reported homology between these two genomes, 16 to 25% of the PMCs were found in anaphase I and II to have either one chromosome bridge or occasionally two chromosome bridges involving different pairs of chromosomes. Unequal distribution of chromosomes at anaphase I (12:10) was also observed. The analytical data indicated that at least two large paracentric inversions differentiated the two species in the hybrids studied. The high crossover rate within these inverted segments

of the chromosomes causes nearly half of the pollen abortion rate observed in the interspecific hybrids studied.

An easy method of staining prophase chromosomes in scarlet runner bean (Phaseolus coccineus) was developed by applying a small quantity of 45% iron propionic carmine for a longer than conventional treatment time. By using this staining procedure, it was found that the 11 pairs of chromosomes could be identified in the diplotene stage by means of characteristic banding patterns and the relative chromosome lengths. The 11 pairs of chromosomes were presented individually with photomicrographs, interpretive drawings, and an idiogram. The most suitable techniques for flower bud collection, slide preparation, and photomicrographic illustration of meiosis were developed and presented in detail.

INTRODUCTION

Common bean blight is a very serious bacterial disease in tropical regions where beans are grown in the rainy season under high temperatures. No effective control measures for this disease are available once the region is infested. No common bean variety is reported to contain resistant genes against tropical strains of this bacterial disease. However, genotypes of Phaseolus coccineus have been selected at the Mayaguez Institute of Tropical Agriculture in Puerto Rico for blight resistance in an environment favorable for bacterial infestation (Dr. Vakili, personal communication). However, the scarlet runner bean lacks many of the essential qualities of the common bean (Phaseolus vulgaris).

The transfer of genes for resistance from P. coccineus to P. vulgaris is a major objective of the bean breeding program at the University of Florida at Gainesville. However, despite the reported chromosome homology between these two species, the interspecific F_1 hybrid plants are usually semi-sterile. The genetic study of the progenies is difficult because of selective embryo abortion, leading to selection against certain genotypes. Therefore, a cytogenetic investigation was conducted to search for abnormalities during meiosis in the F_1 hybrids to increase basic knowledge of Phaseolus cytogenetics.

Early attempts to stain and identify bean chromosomes (14,24) failed because the prophase of mitosis did not offer sufficient differences in chromosome morphology. An effective staining technique is

needed to enable researchers to examine early prophase chromosomes in bean species (24). If the prophase stages of meiosis in bean could be adequately observed, it might be possible to identify the 11 chromosomes of bean cytologically.

PART I

MEIOSIS IN COMMON BEAN (Phaseolus vulgaris)

INTRODUCTION

The meiotic sequence in maize (27) and in tomato (2) has been published for analytical purposes. However, the meiotic sequence in common bean and other Phaseolus species has not been published with proper photomicrograph and idiogram illustration. Publications covering bean cytogenetic analysis are scarce partly because of the difficulties in staining chromosomes in the prophase stage (24).

The importance of meiotic configurations is presented by the following arguments:

1. Knowing the whole sequence in advance is very helpful in planning a cytogenetic research program and determining which stage to investigate in order to get the desired data. The desired stages may not be observable or may not be sufficiently clear to fulfill the experimental purpose.
2. When the cytogenetic data are in disagreement with reports of work with other species, differences in meiotic sequence may help to interpret the discrepancies.
3. Detailed knowledge of the meiotic sequence enables one to judge the suitability for cytogenetic research offered by the species in question. By comparing the meiotic configurations of maize with those of beans, it is known readily that the chromosomes of maize exhibit greater detail than those of beans. Bean chromosomes are so small at metaphase that the fragmentation produced by chromosome aberrations can

not be detected. Such fragments were essential to the precise cytogenetic analysis made by McClintock in maize (21). Double chromosome bridges involving one chromosome pair are easily detected in maize but not in beans.

The presentation of meiotic configurations in beans helps researchers to understand the advantages and the limitations of cytogenetic studies in this species, avoiding useless speculation.

In this paper, we present meiotic configurations in bean PMCs from interphase to pollen mitosis in photomicrographs taken at 500 X and more often at 1000 X, using the phase contrast system in most of the pictures. Discussions were made of the meiotic sequence itself, of staining procedure innovations, and of the problems encountered in interpreting the PMCs observed.

LITERATURE REVIEW

The classical scheme of meiotic sequence was established around 1930. The order of interphase - prophase (leptotene - zygotene - pachytene - diplotene - diakinesis) - prometaphase I - metaphase I - anaphase I - telophase I - interkinesis - prophase II - metaphase II - anaphase II - telophase II - tetrad - pollen grain was followed by Rhoades in his presentation of meiosis in maize (27). A brief discussion of the observations of each stage was also presented in his paper. However, molecular genetics began to be developed after Wastson and Crick published the physical structure of DNA in 1953. This was a long time after the publication by Rhoades (27). Therefore, some of his interpretations and conclusions did not agree with reports of meiotic events studied at the molecular level, especially his interpretation of the structure and function of the nucleolus. Nevertheless, his meiotic illustrations were very helpful, and many textbooks adopted them for use in their chapters describing meiosis.

Brown (2) studied the function of euchromatin and heterochromatin in chromosome #2 of tomato during meiosis. He tried to determine whether these two kinds of chromatin are different in the timing of pairing, chiasma location and condensation rate. He found that chromatic zones (heterochromatin zones) pair more slowly than centromeric and achromatic zones (euchromatin zones) from zygotene to pachytene. Only 30% of the chiasmata terminalize in the long euchromatin arm at

diakinesis while the average terminalization percent of all tomato chromosome arms is 70%. In the short heterochromatic arm of chromosome #2, chiasmata also occur at a much lower frequency. He concluded that the heterochromatic zones at both sides of the centromere determine the chromosome morphology at metaphase.

The tomato chromosome map was established by Barton (1), using the distinct heterochromatic structure at pachytene on both sides of the centromere to differentiate the 12 chromosomes of tomato. The heterochromatic zones are so specific that they compensated the dimensional error produced through camera lucida drawings made during chromosome identification.

The pachytene banding technique was used by Krishnan and De (14) in an attempt to establish a chromosome idiogram for the Phaseolus aureus genome. Unfortunately, the heterochromatic bodies in bean were not as distinct as in tomato. In addition to experimental error, their chromosome diagrams made at pachytene and metaphase led to many problems of interpretation.

The classical scheme of meiosis was established on the basis of PMCs observed under the microscope. Two of the events are challenged today although the challengers do not have the type of direct proof to change the "conventional wisdom" represented by the classical scheme.

One of the challenges is that chromosome pairing occurs at premeiotic mitosis. Therefore the pairing at zygotene in the classical scheme is pre-determined. The formation of a synaptonemal complex is not a pairing process but rather the production of a device for crossing over. The indirect proof comes from fungi, animals and plants (3) where

chromosome pairing is observed before leptotene stage. The challenge, however, does not alter the scheme of meiosis because at early prophase or interphase cytogenetic techniques do not have the necessary power of resolution to settle the question directly.

Another challenge is more serious, and an alternative scheme has been proposed to substitute for the classical one. It was discovered by Moens (23) that there is a meiotic time sequence in the tomato anther. The PMCs located at the basal part of the anther divide first and those at the tip divide later. Thus in a longitudinal section, the PMCs show the meiotic sequence from the tip to the basal part in the order of occurrence. In this time sequence it was found that below the pachytene zone, instead of the expected diplotene cells, the zone is filled with PMCs having diffuse chromatins similar to the leptotene and zygotene chromosome structure. Below this zone, the chromosomes begin to condense again into diplotene, diakinesis and the remainder of the classical scheme. Does zygotene come after pachytene is the challenge. There is a question about the adequacy of the interpretation given to the meiotic sequence in the classical scheme. Until direct proof can settle the matter, the challenge provides an alternative view of the matter.

MATERIALS AND METHODS

The Phaseolus vulgaris varieties La Vega, Great Northern #1 sel. 27, and a breeding line, 6-19, were used for this study. The flower buds were harvested in the morning at about 9:30 a.m. and fixed in Carnoy's fixative containing 6:3:1 by volume of ethanol(95%):chloroform:glacial acetic acid. After 24 hours of fixation, the buds were transferred to 70% ethanol for preservation. A dissecting microscope was used to extract the anthers from flower buds between 2.5 and 3.0 mm in length. The extracted anthers were put in an excess of 45% iron propionic carmine (2.0 ml for every 50 anthers) for one hour to stain the chromosomes of all the stages except prophase. To observe prophase chromosomes, a batch of 50 anthers was placed in 0.5 ml of the same stain for one hour. During this period, the stain dries to 1/3 of the original volume, which creates a stain concentration gradient. These higher concentrations are needed to adequately stain prophase chromosomes.

Bean chromosomes are much smaller than maize chromosomes. In order to have better chromosome spread, a more flattened PMC preparation is required. In fact, mounting technique is a critical factor in bean cytogenetics. From our experience of preparing hundreds of slides, the best mounting procedure is described in detail below.

After staining, the anthers in the porcelain well were transferred to the slide, using a disposable pasteur glass pipet 14.60 cm long. The anthers were sucked in and carried with the stain solution

in the narrow region of the pipet near the tip. If the anthers are sucked into the broad chamber of the pipet, they cannot be discharged easily.

After the anthers were transferred onto the slide, the next step is to check the quantity of stain. Squashing the anthers in an excessive amount of stain will expel many of the PMCs when the cover-glass is put on. Therefore, the quantity of stain should be controlled to the amount just enough to flow when the slide is tilted. Any excess dye should be removed by absorption with an absorbent material such as Kimwipes or filter paper.

Squashing the anthers with an ultrafine tweezer #5 should be performed until no visible tissue pieces are present. Generally it takes two minutes to reach this stage. Too much squashing will damage the larger PMCs which are so precious for bean cytogenetic studies. In order not to spread the PMCs out of the range of the coverglass, the squashing is done with all the anthers concentrated at the middle of the slide. The larger fragments of debris should be eliminated as much as possible before the dye dries excessively. Coagulation of squashed materials will occur if the stain concentration is increased too much by evaporation or if a dirty tweezer containing traces of dried materials is used for squashing. When squashing is complete, the drop-let containing the squashed material is then spread out over an area nearly equivalent to the size of the coverglass. However, the squashed material should not be spread to the perimeter of the coverglass because the PMCs will be lost during blotting. Another reason to restrict

the squashed material is that the edge of the coverglass will be sealed with sticky wax. Therefore, all the PMCs near the edge are not available for observation. A 2-mm border around the coverglass should be left free of the squashed materials.

After the coverglass is put on the squashed materials, the slide is blotted between two pieces of filter paper to get rid of excessive stain and small debris. Big PMCs are flattened during this step and remain under the coverglass.

After blotting, the coverglass is sealed with sticky wax while maintaining a high compression force applied on the coverglass with a cork stopper. These operations are done simultaneously. One hand presses the cork stopper on the coverglass and the other hand seals the coverglass with a curved dissecting needle and hot wax. The high compression force helps to make the PMCs flat. Additional stain is placed under the coverglass when air bubbles or air space are present. The ultrafine tweezer #5 is good for this "filling" work. A good slide should not have air bubbles sealed within, because they will shorten the life of a temporary slide by absorbing the moisture from the PMCs.

Fresh slides were used for photomicrographs using 500 X and 1000 X magnification. The phase contrast system was used to photograph most of the stages. Kodachrome slide film with ASA 25 and black and white film Panatomic-X with ASA 32 were used for photomicrographs.

Because of the unavailability of PMCs containing chromosome bridges and laggards in *P. vulgaris*, the PMCs from hybrids of *P. vulgaris* x *P. coccineus* were used to make photomicrographs (Figs. 28, 29, 41, 48) of such irregularities.

RESULTS AND DISCUSSION

Slide Preparation

The preliminary problems that a cytogeneticist encounters before he begins to work on a certain species of plant are:

a) determining the time of occurrence of meiosis in the anthers during a 24-hour day, b) how to obtain large PMCs with their chromosomes well spread, and c) how to obtain maximum contrast between chromosomes and cytoplasm through the process of slide preparation.

In cytogenetic work, photomicrography is the principal tool for presenting the results of the research. Good quality pictures can only be obtained from good quality slides. Even for a cytogeneticist with long experience, the above three problems should be solved for every new species before he starts to work on any problem.

The duration of meiosis varies from plant to plant. In Ornithogalum virens (Lilliaceae), it was reported that it takes 4 days to complete a meiotic cycle at 18°C (4). However, in most of annual plant species the duration of meiosis is not so long. In these plants, most cytogeneticists harvest the flower buds in the morning between 9:00 a.m. and 11:00 a.m. Since it is easy to obtain dividing PMCs at this time, the duration of meiosis in a plant species is generally neglected by the researchers.

No information about the duration of meiosis in beans was available. Therefore a survey was performed on 10/1/78 to determine

the time of meiosis in this species. Buds were sampled every 30 minutes from 8:00 a.m. to 2:00 p.m. It was found that the major peak of meiotic activity occurred between 9:00 a.m. and 11:00 a.m. The buds collected before or after this period contained very few PMCs in metaphase and anaphase. The results indicate that bean meiosis does not last more than 2 hours. The collection of buds before sunset (5:00 p.m.) also contained PMCs in intense meiotic activity. It seems that bean meiosis has more than one activity peak per day. Some PMCs divide in the morning and others divide in the afternoon. It is assumed that meiosis is a function of solar activity in this species.

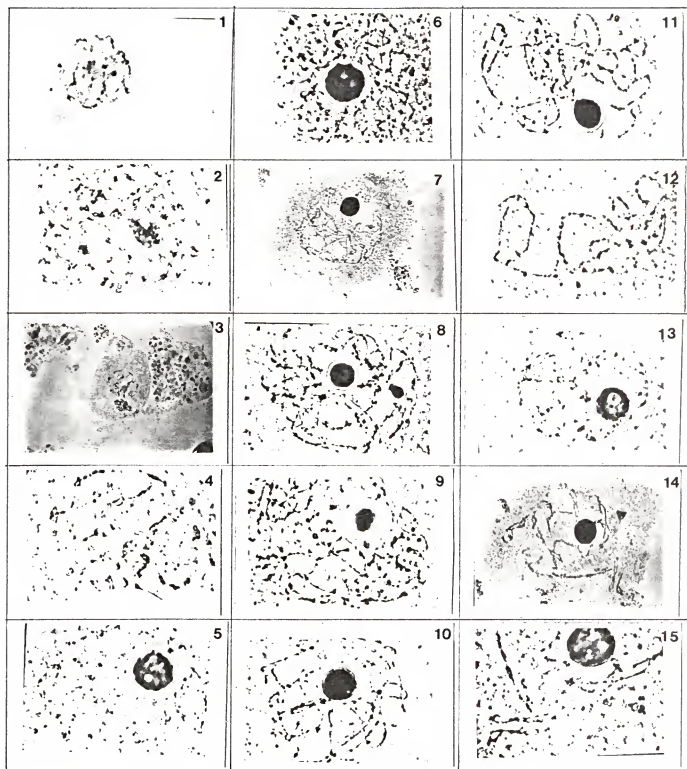
During our work, we discovered that the size of a bean PMC is not constant throughout the flowering period. The larger PMCs were found in anthers collected at the beginning of anthesis and the size diminished as flowering progressed. The large PMCs observed were almost twice the size of small PMCs. However, the size of their chromosomes in meiotic phases is not proportional to the size of PMCs. Larger PMCs are better for observation of prophase chromosomes because they provide more space for the longer chromosomes and reduce overlapping. However, for photomicrographic purposes, smaller PMCs are desirable for those stages after metaphase II because the chromosomes are in widely separated groups in these stages. It is difficult to photograph the whole PMC under 1000 X magnification if the size of PMC is too large. A PMC can be photographed at 500 X magnification, but the chromosome detail is not as good as at 1000 X. Photographs containing the whole PMC are important for the study of chromosome number, especially for aneuploid study. Under 1000 X or higher magnification, the loss

from the field of view of some portion of a large PMC together with one or more chromosomes can be misinterpreted as an indication of aneuploidy.

The preparation of prophase chromosomes in beans required special attention in bud bleaching. Prophase chromosomes can only be stained adequately when the cytoplasm of a PMC is thoroughly bleached by excessive amounts of fixative agents. When too small amounts of fixative are used to fix the bud clusters, the fixative solution becomes very dark with the breakdown of chlorophyll. The dark solution also darkens the cytoplasm of the PMCs, affecting the quality of the preparations.

Meiotic Sequence

1. Interphase is shown in Figs. 1 and 2, showing the same nucleus at 500 X and 1000 X phase contrast. The genetic materials are seen to be contained in the nuclear envelope. Those chromatic strips are heterochromatin bands of the chromosomes. They are in a condensed structure. The achromatic zones represent euchromatin. According to Comings (5), this kind of genetic material is less condensed than heterochromatin.



Figures 1 through 15. Meiotic sequence of bean (*Phaseolus vulgaris*) from interphase to diplotene. (Figures are discussed in the text.)

2. Pre-leptotene is presented in Figs. 3 and 4, showing the same cell at 500 X and 1000 X phase contrast. In this stage, the heterochromatin zones are apparently smaller than those of interphase. Heterochromatin is reported to duplicate after the duplication of euchromatin (3). The heterochromatic zones are probably in the process of decondensation and duplication. Biochemical data reported by Hotta et al. (10) showed that DNA replication is not completed until the pachytene stage. They showed that any inhibition of DNA synthesis before this stage produces damaged chromosomes. They refused to attribute the late synthesis (after leptotene) of DNA to the repair mechanism which mends broken chromosome ends during crossing over. In the classical scheme, DNA duplication is thought to be accomplished before leptotene.

3. Leptotene is presented in Figs. 5 and 6, both at 1000 X phase contrast. The nucleolus is the only distinct body. The volume of the nucleus is greatly increased, and the chromatin threads cannot be seen clearly in this stage.

4. Zygotene is presented in Figs. 7 through 11 in which the homologous chromosomes synapse gradually. According to Brown (3), each chromosome synthesizes a "lateral element," a proteinaceous core structure along the side to be synapsed later. When the lateral elements of the two homologous chromosome segments are put in juxtaposition, a proteinaceous "central element" is formed parallel to the orientation of the chromosome segment. Transversal fibrils are formed to connect lateral and central elements. Thus, the "zippering" of the two homologous segments is accomplished. The whole structure is called

a synaptonemal complex. The lateral element is thought to recognize the homolog at juxtaposition for synapsis. The lateral element has a width of 400 A° along side the chromosome segment. The central element is 160 A° wide. The transversal fibrils are about 500 A° long at each side. Thus, the distance between the two synapsed chromosome segments is about 2000 A°.

Under a light microscope, 2000 A° is the minimum dimension that can be seen. Therefore, the synaptonemal complex between the two chromosomes is seen as a thin line. Its fine structure, as mentioned above, was discovered through electron microscopy.

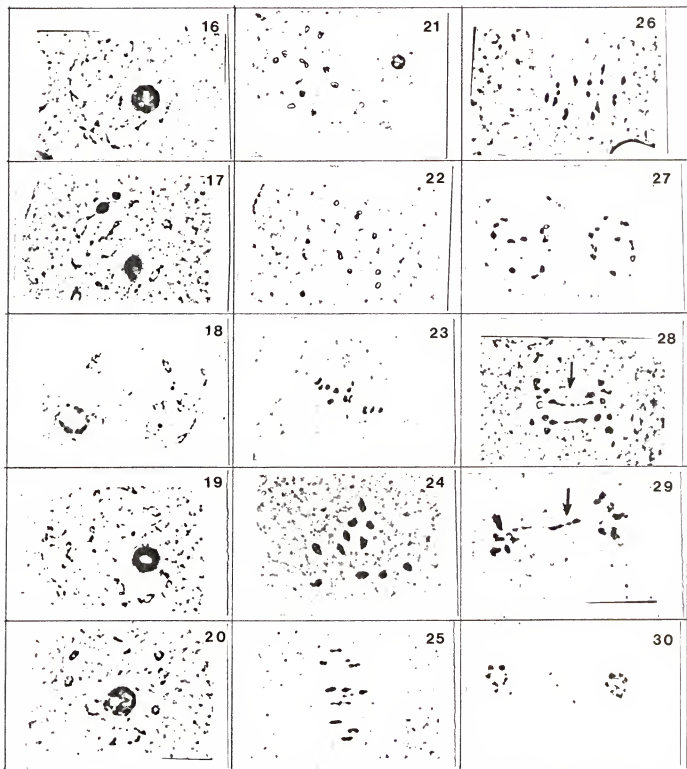
The synaptonemal complex is directly involved in crossover events. Chromosomes that pair without a synaptonemal complex (as in mitotic pairing, or due to deletion or mutation) have fewer chiasmata. The protein subunits for assembling the synaptonemal complex are synthesized only at this stage during meiosis.

5. Pachytene is presented in Fig. 12 in which the two homologous chromosomes are completely united by the synaptonemal complex. Unlike tomato pachytene, the pachytene chromosomes of beans do not have distinct and thick heterochromatic regions at both sides of the centromere. The configurations of P. vulgaris from mid-zygotene to pachytene show that the heterochromatic bodies are well distributed along the chromosome arms. The structure of P. vulgaris chromosomes has no similarity with that of P. aureus as reported by Krishnan and De (14). During the prophase stage before diakinesis, most PMCs in P. vulgaris present only one spherical nucleolus. However, 3.4% of the 115 PMCs studied contain one or more extra nucleolus-like bodies

attached at different chromosomes as shown in Figs. 8, 17 and 34. In P. coccineus we found that 18.2% of the 235 PMCs observed contain these extranucleolar bodies. In the hybrid of P. vulgaris x P. coccineus, we found that 22.1% of the 285 PMCs observed contain extra nucleolar bodies. The hybrid possesses the sum of the frequencies of the extranucleolar bodies observed in the two parental species. The extranucleolar bodies are smaller than the normal nucleolus although the shape and chromatic nature are similar.

The nucleolus is an active transcribing section of the chromosome during prophase. It is composed of puffed DNA coils and the transcribed ribosomal RNA molecules. The ribosomal RNA molecules diffuse from the nucleolus to the cytoplasm to combine with protein components, forming ribosomes which are the site of protein synthesis (translation)(3). In the meiotic prophase of amphibians, many small extranucleoli not associated with chromosomes are seen floating within the nucleus. They are synthesized through a special DNA multiplication process called gene amplification in which copies of DNA from the chromosomes are released into the nucleus for a quick transcription of ribosomal RNA in order to translate a large quantity of proteins in amphibian egg cells (3).

Multiple nucleoli are also found in polyploid plants (3). The presence of extranucleoli in some fraction of bean PMCs seems to have evolutionary significance. It looks as if genes located in the primary nucleolus may be duplicated at sites in other chromosomes, and these genes are normally activated only in a fraction of the PMCs. One may speculate that such redundancy of genetic information provides a "safety factor" against the possibility of mutations or deletions of the principal gene locations in the primary nucleolar chromosome.



Figures 16 through 30. Meiotic sequence of bean (*Phaseolus vulgaris*) from diplotene to telephase I. Figures discussed in the text; Figs. 28 and 29 were taken from hybrids of *P. vulgaris* x *P. coccineus*.

At the pachytene stage, inversion loops, addition-deficiency loops, and translocation pairing can be observed if such chromosomal aberrations exist in an aberration heterozygote.

When a pachytene chromosome is in a tilted position, it is difficult to measure its absolute length and structure. Measurement of chromosome length in pachytene has limited value in bean cytogenetic analysis and is probably unfeasible due to excessive overlapping.

Although metaphase chromosomes of many species of animals and plants are easily identified, pachytene morphology is used in maize (27) and tomato (1) for chromosome identification. Their pachytene chromosomes possess distinct physical markers. Unfortunately, in beans such markers are not conspicuous enough to be used for chromosome identification.

There are ambiguous situations in pachytene interpretation when some sections of the chromosome pair remain open as rings. It is difficult to tell if the failure of synapsis is due to an inversion, an addition, a deficiency, or simply a delay in the synaptic process. Therefore, pachytene observation is a supplemental approach rather than a major one in the study of translocation, inversion, or addition and deficiency.

6. Diplotene is presented in Figs. 13 through 19 in which the chromosomes are condensing and separating. Chiasmata appear at the location of crossovers and move to the ends of the chromosomes through the process of terminalization. The centromeres are paired during this stage (Fig. 14). In beans, this is probably an ideal stage for chromosome identification because the chromosomes are more spread.

The characteristic banding patterns are still present. The two homologous chromosomes are located side by side, which facilitates comparison by observing details of the banding sequence. Any pair of chromosomes which does not have any crossovers does not have any points of contact, and the members of the pair may even be widely separated.

7. Diakinesis is presented in Figs. 20 and 21. At early diakinesis the chromosomes are thick, with chiasmata terminalized at the chromosome ends. Many chromosome pairs appear as rings because the centromeres are no longer synapsed, and the chiasmata in both arms are terminalized. Some chiasmata are not yet terminalized at this stage.

Late in this stage, the nucleolus appears to be reduced in size. This is the stage of maximum chromosome condensation.

When there is insufficient homology between the two related chromosomes, the univalents are often widely separated at diakinesis.

8. Prophase chromosomes are the same as metaphase I chromosomes. However, they have not migrated to the equatorial plate yet. The chromosomes in Fig. 22 may be at this stage. They could also be in another orientation of metaphase I chromosomes.

9. Metaphase I chromosomes are present in Figs. 23 through 26 in which the chromosomes are located at the equatorial plate. Each centromere faces each pole. The inconspicuous spindle fibers begin to pull the chromosomes to each pole. It is difficult to identify bean chromosomes at this stage because most of the chromosomes are not in longitudinal orientation (Fig. 23). Some pairs of chromosomes may separate earlier than others. The event (Fig. 26) is called "precocious separation."

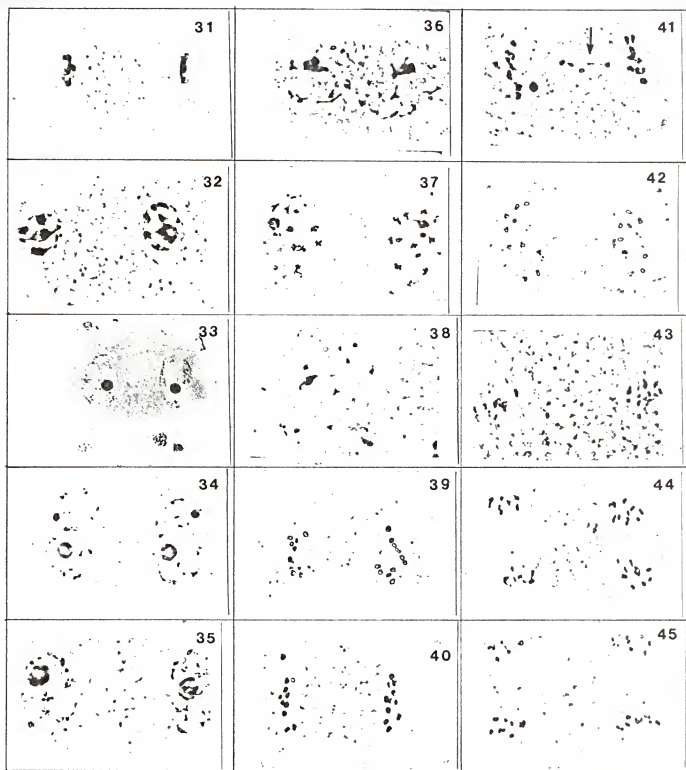
10. Anaphase I is presented in Figs. 27 through 29 in which the chromosomes are migrating to each pole. A proteinaceous matrix begins to connect the chromosomes into a spherical mass. Certain types of crossover events occurring within the inversion loop (3,21) can give rise to the chromosome bridges shown in this stage (Figs. 28 and 29); when chromosome bridges appear in high frequency, pollen sterility is also high. The quantitative relationship between the bridge frequency and the pollen abortion ratio is discussed by McClintock (21) and Brown (3).

The acentric fragment produced during chromosome bridge formation cannot be detected in most of bean PMCs (Figs. 28 and 29). The inability to detect acentric fragments greatly reduces the power of bean cytogenetic analysis to resolve important questions.

11. Telophase I is presented in Figs. 30 and 31, in which the chromosomes are organized into a spherical mass. Euchromatin zones begin to diffuse the chromatin into invisible fibrils. Then the two daughter nuclei are formed.

Unlike maize PMCs, bean PMCs do not form a cell wall between the two daughter cells at the end of the first division. Therefore, the chromosome bridges in beans are not cut by the cell wall at this stage. Many bridges are left intact at metaphase II.

12. Interkinesis is presented in Fig. 32. The nucleolus reappears in each daughter nucleus, and the heterochromatin zones are thick and more compact than those at interphase before meiosis. The transcription of RNA in the euchromatic region is active in this stage.



Figures 31 through 45. Meiotic sequence of bean (*Phaseolus vulgaris*) from telophase I to anaphase II. Figures discussed in the text; Fig. 41 was taken from a hybrid of *P. vulgaris* x *P. coccineus*.

13. Prophase II is presented in Figs. 33 through 38. This process basically repeats the prophase I process mentioned above, except that no DNA replication occurs at early prophase II. The daughter nucleus in prophase II is much smaller than that of prophase I. Therefore, their chromosomes are not easy to identify although details of chromosome morphology are sharp and clear in some PMCs.

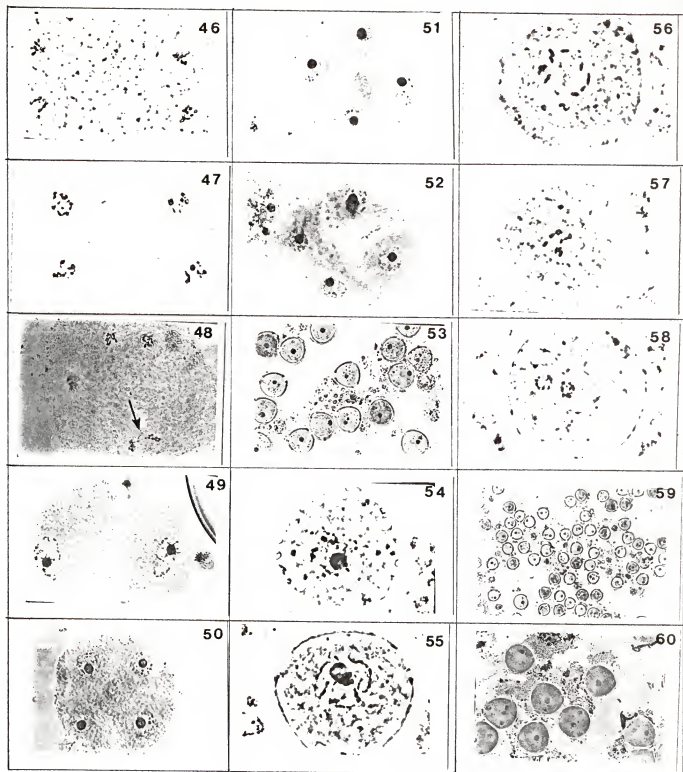
14. Metaphase II is presented in Figs. 39 through 42. The metaphase chromosomes are spread on two equatorial plates in the two daughter cells of the PMC.

Although they appear as two groups of chromosomes at the polar regions, similar to that of an anaphase I PMC configuration, they do not have a proteinaceous matrix connection. They are not subject to the pulling force of the spindle fibers which fold the anaphase I chromosomes. They are relaxed on the equatorial plates waiting for further division. The doubleness of the chromosomes can be seen in Fig. 42.

If the chromosome bridge does not break at anaphase I, it will show up in this stage as a narrow thread with fragments near the bridge (Fig. 41). If the bridge breaks, many laggard chromosomes are seen floating between the two groups of chromosomes.

15. Anaphase II is presented in Figs. 43 through 46 in which the chromosomes split and migrate to the four poles within the PMC.

Chromosome bridges (Fig. 48) appear in this stage if there have been two crossovers during the first division, one occurring within the inversion loop and the other in the proximal region, involving three of the four chromatids. Many laggard chromosomes also appear in this stage.



Figures 46 through 60. Meiotic sequence of bean (*Phaseolus vulgaris*) from anaphase II to pollen mitosis. Figures discussed in the text; Fig. 48 was taken from a hybrid of *P. vulgaris* x *P. coccineus*.

16. Telophase II is presented in Fig. 47 in which the chromosomes of each pole are connected by the proteinaceous matrix. Four nuclei are formed within the PMC without the formation of cell walls to separate them.

If a chromosome bridge breaks late in this stage, the nucleus containing the broken chromosome will show a protrusion toward the opposite nucleus. Since no cell wall is formed, many bridges remained intact.

17. The tetrad stage is presented in Figs. 49 through 51. The four nuclei within the PMC develop from outside inward to separate the PMC into four equal parts, each part having a pyramid shape (Fig. 51) and containing a nucleus. The heterochromatin regions and the nucleolus can be seen in the nuclei.

18. The pollen stage is presented in Figs. 52 through 60. The tetrad cells change from their pyramid shape into a spherical shape within the PMC envelope (Fig. 52). The pollen grains are released with the rupture of the envelope (Fig. 53). Each pollen grain presents 4 exines (pollen tube exits) at the four corners.

The nucleus of a pollen grain undergoes two mitotic divisions (Figs. 54 through 60), producing three nuclei in the pollen. One nucleus functions in pollen as the tube nucleus, one fertilizes the egg nucleus to form an embryo, and the other fertilizes the two polar bodies to form a triploid endosperm. According to Weinstein (33) the endosperm of bean develops first during seed development, and all of its materials are absorbed by the two cotyledons during the later stages of seed formation.

During pollen mitosis, the nucleus contains only 11 chromosomes. In many nuclei, they are well spread. Nevertheless, it is difficult to

use pollen cells for chromosome identification because the chromosomes are very small. Frequently, the pollen nucleus cannot be seen clearly because of the presence of starch grains in the pollen.

In Fig. 60, pollen grains with one, two and three nuclei are present in the pollen population. However, on the day of anthesis, the pollen nuclei are not visible after staining with iron propionic carmine for 10 minutes. Mature pollen appears turgid and spherical in shape. The shrunken pollen and empty pollen can easily be distinguished from normal pollen filled with cytoplasm. Pollen analysis for semi-sterility is an important part of cytogenetic analysis.

SUMMARY

A series of photomicrographs illustrating the entire sequence of meiosis in common bean (Phaseolus vulgaris) was presented to discuss the role of each stage in the cytogenetic analysis of beans. The peculiarities of each stage were presented and their implications in bean cytogenetic analysis were discussed. The study of pachytene PMCs reported here is far from complete. Nevertheless, it is clear that heterochromatin is well distributed throughout most of the chromosomes. This contrasts markedly with tomato chromosomes in which the heterochromatin is located in the proximal regions on both sides of the centromeres while the distal regions are largely achromatic.

In the common bean, the identification of the individual chromosomes can best be achieved at diplotene because of the distinctive banding patterns. Although chromosome bridges resulting from inversions can be seen at anaphase I and anaphase II, the double bridges in one chromosome and acentric fragments reported by McClintock (21) in maize are not observable in common bean.

PART II

CYTOGENETIC ANALYSIS OF INTERSPECIFIC HYBRIDS BETWEEN
COMMON BEAN (Phaseolus vulgaris) AND THE
SCARLET RUNNER BEAN (Phaseolus coccineus)

INTRODUCTION

High pollen abortion has been observed previously (16,17) in the F_1 plants from crosses between Phaseolus vulgaris and Phaseolus coccineus. However, the meiotic process of the F_1 plants was reported to be normal and without any evidence of chromosome aberration. The two genomes were reported to have identical chromosome structure on the basis of Giemsa staining of root tip chromosomes (24). This banding technique is presently the only means of identifying the 11 chromosomes of beans (25). There is still no satisfactory explanation of the mechanism of pollen abortion in the hybrid plants (16,17,18,30).

The only report about abnormal meiosis in F_1 plants was published by Xolocotzi et al. (34) in Mexico. In natural interspecific hybrids of these two species, 25 to 50% of pollen mother cells (PMCs) presented a chromosome bridge in anaphase I and anaphase II. Two univalents in prophase stage were observed occasionally. Although such high meiotic irregularity was present, these authors did not report pollen viability or seed development in their paper. The partial restoration of pollen fertility in amphidiploids derived from these two species reported by Smartt and Haq (30) implied the existence of gross chromosome structural differences between these two genomes, besides the presence of genic sterility.

Evidence of interspecific barriers between P. vulgaris and P. coccineus is observed frequently in such problems as difficulties in

obtaining F_1 seeds (11,16), the failure of F_1 plants to develop, the failure of F_1 plants to flower (13), high sterility in F_2 and advanced generations, and the loss of desirable genes from P. *coccineus* (19,32). Although environmental conditions may contribute to pollen abortion, no previous study relates inversions with the high percentage of pollen abortion in interspecific hybrid beans.

In this paper, we have searched for meiotic aberrations to explain the high pollen abortion observed and have challenged previous reports that the chromosome structure of these two species is probably free from large aberrations.

LITERATURE REVIEW

The genetics of hybrids between P. vulgaris and P. coccineus was studied thoroughly by Lamprecht during the 1940s (16,17,18,19). In his studies of the inheritance of more than a hundred characters differentiating these two species, he concluded that only two characters can neither be transferred from P. coccineus to P. vulgaris nor vice versa. The two characters are the cotyledon position and stigma orientation. P. vulgaris has an epigeous cotyledon and an internal stigma position. P. coccineus, on the other hand, has a hypogeous cotyledon and an external stigma position. The former species is self-pollinated while the latter is cross-pollinated by insects. These two characters ("interspecific genes" according to Lamprecht) are thought to be specific for each species. It was postulated that some unspecified type of isolation mechanism prevents the incorporation of species specific genes into another species.

Lamprecht observed chromosome pairing in the hybrids from these two species and found the 11 bivalents at diakinesis and metaphase I to be normal. Two univalents occur only occasionally. In his reports Lamprecht stressed that the meiotic process of the hybrid runs a normal course without detectable aberration. However, he did not mention how many PMCs he analyzed at anaphase I, anaphase II or metaphase II. His publications indicate that he scanned many cells at diakinesis and metaphase I and found them all normal. Therefore, the

examination of the later stages probably was not conducted in detail before he ruled out the possibility of any structural differences between the two sets of chromosomes.

In order to explain the occurrence of a 60-80% pollen abortion rate, he set up the hypothesis that specific genes of P. coccineus cannot be replicated in P. vulgaris cytoplasm. During the reproductive process in the hybrid there is selective suppression of replication of certain alleles from P. coccineus. These specific alleles from P. coccineus are thus eliminated gradually as the inbreeding process approaches homozygosity. He called this hypothesis "mutation of inter-specific genes." This hypothesis involves selective replication of DNA (copy choice) which is inconsistent with current molecular genetic principles.

Mok and Mok (24) tried to detect the structural differences between the chromosomes of P. vulgaris and P. coccineus through Giemsa staining at the mitotic prophase stages. They reported that no difference could be detected using this technique. These results are in agreement with the opinion of Lamprecht that no chromosome structural differences exist between these two species. They also used this technique to identify monosomic chromosomes in P. vulgaris (25).

Chromosome structural differences within P. vulgaris were reported by Honma (9). He found that at least two inverted segments are present in different chromosomes in crosses between Blue Lake pole bean and Contender. The supporting evidence was the repeated observation of two chromosome bridges present simultaneously in different pairs of chromosomes in the same PMC. Honma suggested that these inversions may

explain his failure to transfer pole Blue Lake pod characters into the bush bean Contender.

An inheritance study of cotyledon position in hybrids between these two species was conducted later by Wall and York (32). They found that the inheritance of this character is quantitative and that both the hypogeous cotyledon and external stigma gene frequencies decrease progressively with successive inbreeding generations.

Innes (11) reported the complete failure to backcross the interspecific hybrid onto the P. coccineus parent used as female. His breeding program encountered many difficulties because he used P. coccineus as female. Lamprecht (16,19) had reported earlier that P. vulgaris must be used as female parent for interspecific crosses.

Kedar and Bemis (13) reported arrested development among some fraction of interspecific F_1 plants. The behavior of these dwarfs varies from dying in the seedling stage to developing a weak and sterile plant. Two different physiological blockage factors were found in different hybrids. When the two factors are genetically combined into one genotype, the resulting seedling is arrested in development with the cotyledon energy only partially used. The seedling dies within a few weeks with the cotyledons largely unabsorbed by the plant.

A detailed analysis of chromosome inversion in maize was published by McClintock in 1938 (21) using an inversion in chromosome #4 for quantitative study. Since the acentric fragment produced by crossing over within the inversion loop is visible in the cytoplasm during meiosis and pollen mitosis, she was able to observe the fate of these fragments and conclude that such a fragment is randomly distributed

into the four spores derived from a PMC. From counts of sporocytes with bridge configurations at the second division, she estimated that 75% of the anther spores contain the normal chromosome #4 while 25% contain the fragmented chromosome. The frequency of a normal spore ending up with an acentric fragment was determined to be 7.5% while the frequency of an aberrant spore (carrying a fragmented chromosome #4) getting an acentric fragment was 26%. By counting the bridged chromosome frequencies in the pollen grains during the mitosis and interphase stages, she found that most of the pollen in mitotic phase did not have bridge protrusion and that those pollen cells with bridge protrusion were still in interphase. She concluded that pollen containing a fragmented chromosome exhibits delayed pollen mitosis. During the anaphase stage of pollen mitosis, she observed that the bridge frequency was the same as the frequencies of chromosome bridges during anaphase I and anaphase II in meiosis. Thus, she concluded that the chromosome bridges observed in pollen mitosis are derived from chromosome bridges in anaphase I and II which broke apart. The broken ends of the fragmented chromosome and its newly replicated copy are joined together during pollen mitosis. A bridge is formed when the centromeres are pulled to the poles.

In beans, the cytogenetic analysis is not as precise as in maize because the chromosomes are much smaller in most meiotic stages and their fragments are not detectable in most of the PMCs. However, the mechanism of chromosome bridge formation and its consequences in maize also apply in beans.

In her diagram of chromosome bridge formation, she showed the types of crossovers that produce:

1. Single bridge at anaphase I with one acentric fragment.
2. Double bridge with two acentric fragments at anaphase I.
3. Single bridge at anaphase II.
4. Double bridge at anaphase II.

A more complete mechanism of chromosome bridge formation was presented by Brown (3). He cited five types of crossover events that give rise to an anaphase I bridge:

1. Single exchange in the inversion loop.
2. Three-strand double in the loop.
3. Two or four-strand doubles with one in the loop and one in the proximal region.
4. Three-strand double with one in the loop and one in the distal region.
5. Four-strand double in the loop (double bridges).

Only one type of crossover gives rise to an anaphase II bridge, viz., the three-strand double with one crossover in the loop and one in the proximal region.

Chromosome bridges can be formed with a higher number of crossovers. The frequency of such cases is so low that it is negligible. One or two crossover events within a chromosome are usually taken into consideration in analytical data.

Chromosome bridge formation produces pollen abortion. During bridge formation an acentric fragment, which carries the duplicate distal sections and the whole inversion loop, is lost in the cytoplasm.

Therefore, both of the two chromosomes lack the distal section after bridge breakage. The random breaking point along the chromosome bridge also produces duplication and deficiency in the fragmented chromosome. Thus the occurrence of an anaphase I or an anaphase II bridge is responsible for the abortion of two of the four pollen grains formed from a PMC. Occasionally the aberrant spore can be functional, but this requires that the acentric fragment happens to be in its nucleus (28).

In maize, the chromosome bridges are broken either by the pulling force of the spindle fibers or by the formation of cellular walls between the daughter cells.

The position of an inverted segment can be determined by recombination frequencies, using the inverted segment as a gene to recombine with gene markers along the chromosome in a series of three-point test crosses. The method is illustrated in maize by Morgan (26).

The change of chromosome structure has been thought to be a driving force of the evolution of species. In the Phaseolus genus, Sarbhoj (29) reported that P. vulgaris is probably one of the most primitive species of the genus because most of its chromosomes have either a median or submedian primary constriction, while the more advanced species have either a submedian or a subterminal primary constriction. The chromosome breakage and fusion events during the evolutionary process damage many genes at or near the breaking points, and this results in small and undetectable differences among the species of the same genus. The interspecific hybrid may show some degree of sterility of this nature, resulting from cryptic hybridity (29). Another type of hybrid

sterility between species is produced by major structural differences, e.g., translocation, inversion, addition and deficiency.

Crosses involving advanced Phaseolus species, such as P. aureus x P. trilobus (6), or P. lunatus x P. polystachyus (8), have a higher degree of sterility. Major structural differences between these species were detected through a high number of pachytene loops, univalents and multivalents at diakinesis and metaphase, chromosome bridges at anaphase I and anaphase II, and a high pollen abortion rate.

MATERIALS AND METHODS

Two *P. coccineus* lines (Pc-37 and Pc-50) and three *P. vulgaris* lines (6-19, Great Northern #1 sel. 27, and La Vega) were used in inter-specific hybrid crosses, using the *P. vulgaris* lines as female parents. The two *P. coccineus* pollinator lines were obtained from Dr. Nader Vakili at the Mayaguez Institute of Tropical Agriculture in Puerto Rico. These lines were derived from an open-pollinated population which was mass selected for multiple disease resistance, including high resistance to common bacterial blight.

For pollen abortion analyses, flower and bud materials were harvested in the morning, i.e., both freshly opened flowers and buds due to open in approximately 24 hours. The anthers were stained with 45% iron propionic carmine for 20 minutes before squashing on slides. The unstained, transparent, and shrunken pollen grains were classified as aborted. The round, fully developed, and well-stained grains were classified as normal.

For cytogenetic analysis, the F_1 and parental lines were planted in late August 1978 at Gainesville, Florida. During October, the flower buds were harvested and fixed in Carnoy's fixative containing 6:3:1 by volume of ethanol(95%):chloroform:glacial acetic acid. After 24 hours, the buds were transferred to 70% ethanol for preservation. For meiotic study, anthers were extracted from buds in the range of 2.5 to 3.0 mm in length and were stained in 45% iron propionic carmine for 45 to 60 minutes before squashing on slides.

The PMCs were classified individually according to their stage of meiosis and the presence of abnormalities. Although diakinesis and metaphase I data were taken, only anaphase I and anaphase II data were used for quantitative analysis. Metaphase II configurations were especially emphasized because the chromosomes are well dispersed in the PMCs and because they give valuable information about chromosome aberrations and their consequences.

The mature F_2 seeds were harvested from dried pods on several F_1 plants planted in the spring (March) of 1978. They "over-summered" in spite of die-back of the above ground plant parts during mid-summer. These F_1 plants regenerated from the roots in the fall and flowered early enough to produce mature seeds before frost killed them early in December. The harvested seeds were dried and weighed individually in order to study seed development.

RESULTS AND DISCUSSION

Pollen Abortion Analysis

As presented in Table 1, the interspecific hybrids had 58 to 79% of non-stainable pollen grains while their parental lines had less than 2%. Very little difference was detected between the pollen sterility on the day of flowering and one day before flowering. The range of pollen sterility observed in this work is in accord with the reports of Lamprecht (16) and Smartt and Haq (30). The 60 to 80% of pollen sterility observed is thus typical of hybrids between these two species.

Undivided PMCs four times the size of a normal pollen grain were frequently seen among the pollen population of F_1 plants. Such undivided PMCs were not observed among the pollen grains of parental lines. The interspecific hybridization probably interfered to some degree with the meiotic mechanism during pollen formation. Such a phenomenon has been reported in interspecific crosses in Allium (12).

While the stainable pollen grains of the parental lines are uniform in size, those from F_1 hybrids are heterogeneous (Fig. 61-A2). The differences in size may contribute to different degrees of viability and vigor. If viability and vigor are a function of size, this could lead to differential zygotic frequencies and non-Mendelian ratios for genes linked to loci controlling pollen characteristics.

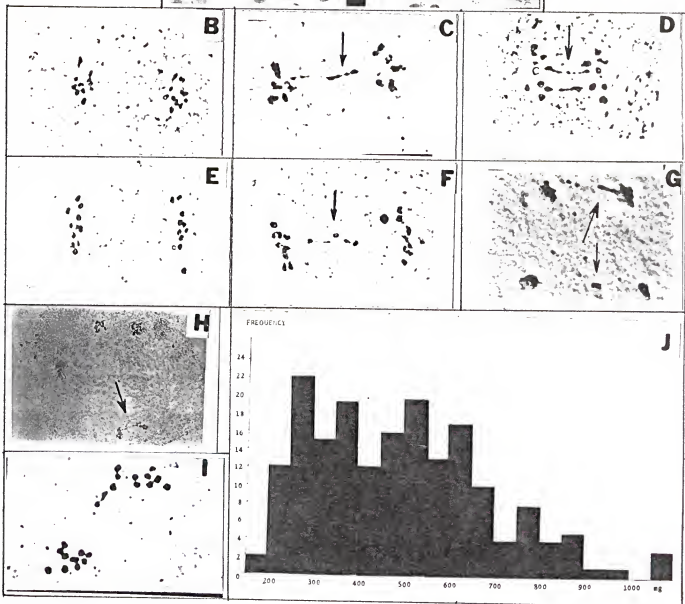
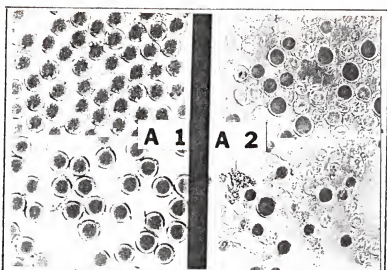
Table 1. Pollen staining analysis at anthesis and one day before anthesis* of *P. vulgaris*, *P. coccineus* (Pc) and their hybrids.

At Anthesis	P. cocc.	P. vulg.	F ₁	F ₁	F ₁
	Pc37	6-19	L.V. x Pc37	G.N. x Pc37	6-19 x Pc37
Total pollen	633	755	1103	586	892
Aborted pollen	13	5	825	342	589
% abortion	2.05	0.66	74.80	58.36	66.03
One day before Anthesis					
Total pollen	651	768	1359	650	664
Aborted pollen	10	6	1078	418	470
% abortion	1.53	0.78	79.32	64.30	70.78

* Anthers were stained for 20 minutes in propionic carmine prior to squashing on slides.

Figure 61. Pollen grains, meiotic configurations of interspecific hybrids between *P. vulgaris* and *P. coccineus*, and a histogram of individual seed weights of interspecific F_2 seeds.

- A-1: Normal pollen grains from *P. coccineus*.
- A-2: Pollen grains from a hybrid plant showing empty pollen and stained pollen grains of different sizes.
- B: A normal anaphase I PMC with two groups of chromosomes arranged in spherical form around each pole (1000 X phase contrast).
- C: A chromosome bridge at anaphase I in a PMC from an F_1 plant (1000 X phase contrast).
- D: Anaphase I PMC from an F_1 plant showing two chromosome bridges joining different pairs of chromosomes (1000 X phase contrast).
- E: A normal metaphase II PMC from *P. vulgaris* showing chromosomes spread on two different planes and a sharp chromosome image (1000 X phase contrast).
- F: A metaphase II PMC from an F_1 plant showing chromosome bridge-laggards (1000 X phase contrast).
- G: An anaphase II PMC from an F_1 plant showing a chromosome bridge in one half and a chromosome laggard in the other half (1000 X).
- H: An anaphase II PMC from an F_1 plant showing a chromosome bridge in one half (500 X).
- I: A metaphase II PMC from an F_1 plant showing 10-12 chromosome distribution (500 X).
- J: A histogram of individual seed weights (mg) of F_2 seeds.



Gross Chromosome Homology Between the Genomes

P. vulgaris and P. coccineus chromosomes share a considerable degree of homology. In diakinesis and metaphase I PMCs, 11 pairs of chromosomes are normally observed in the hybrids of the two species. Two univalents are seen only occasionally, which was also reported by Xolocotzi et al. (34). The low frequency of univalents was also observed in both parental lines. These two univalents appear to be the result of precocious separation of a certain pair of chromosomes. According to the literature, chiasmata are responsible for close pairing at diakinesis and metaphase I. The frequent appearance of precocious separation in one pair of chromosomes probably indicates a low frequency of crossing over in this pair. In interspecific hybrids, asynapsis may occur when structural differences are located along the chromosomes, causing difficulties in pairing. Since the two univalents are also observed at low frequency in the parental lines, the frequency of occurrence of univalents in those interspecific hybrids is not sufficient to indicate structural differences between the two species in the chromosome involved. In other interspecific hybrids of the Phaseolus genus where gross structural differences were reported (6,8), univalents at metaphase I were observed in more than one chromosome and at high frequency.

Although our data on prophase observations cannot detect structural differences of chromosomes, it cannot be used to prove structural homology either. Not all types of structural differences give rise to univalents. Lamprecht (16,18) was not justified in assuming complete structural homology on the basis of prophase observations of the

interspecific hybrids of these two species. However, our data indicate that P. vulgaris and P. coccineus have a closer relationship than that between P. aureus and P. trilobus (6) or between P. lunatus and P. polystachyus (8).

Anaphase I Analysis

The analysis of chromosome inversions was well illustrated by McClintock (21) and by Smith (31). The data from anaphase I and anaphase II are used together to count crossing over events which occurred within the inversion segment. A chromosome bridge occurs either at anaphase I or at anaphase II depending on the type of crossover events involved. According to Brown (3), there are five types of crossover events that produce an anaphase I bridge, but only one type that causes an anaphase II bridge. Their common requirement is that at least one crossover occurs in the inversion loop.

The anaphase I data from our material (Table 2) indicate 15.6 to 25.6% of the observed PMCs have one chromosome bridge, or occasionally they have two bridges in the same PMC involving different pairs of chromosomes (Figs. 61-C and -D). The cross with Pc-50 used as the male parent has a much higher bridge frequency than the crosses with Pc-37 used as male parent. The use of different P. vulgaris lines as female parents did not result in different bridge frequencies.

The high bridge frequencies observed and the simultaneous presence of two bridges at different pairs of chromosomes indicate that there are at least two large inverted segments located in different pairs of chromosomes. These inversions are paracentric.

Table 2. Anaphase I bridge frequencies in P. vulgaris, P. coccineus and their hybrids.

<u>Species</u>	<u>Total cells</u>	<u>1 bridge</u>	<u>2 bridges</u>	<u>% bridges</u>
6-19 (<i>vulgaris</i>)	102	0	0	0.0
L.V. (<i>vulgaris</i>)	50	0	0	0.0
Pc37 (<i>coccineus</i>)	116	1	0	0.8
F ₁ 6-19 x Pc37	143	21	1	16.8
F ₁ L.V. x Pc37	146	21	1	15.6
F ₁ 6-19 x Pc50	117	18	6	25.6

In the intraspecific hybrid of P. vulgaris between the Blue Lake pole bean and the Contender snap bean varieties, Honma (9) also observed two chromosome bridges each occurring in a different pair of chromosomes. His data indicated that two large inverted segments are present, differentiating the chromosomes of the two bean varieties. At the present stage of bean cytogenetics, it is impossible to tell which chromosomes contain these inversions. Also, we do not know whether or not the two paracentric inversions in the present work are identical to the two paracentric inversions reported by Honma. However, the latter question could be answered by crossing line Pc-50 to Blue Lake pole bean and Contender. If the two inversions are identical, one cross should give two bridges in some PMCs and a much higher frequency of bridge-laggards similar to the present work. The other cross should not give any bridges.

The number and size of inversions and the chiasma frequency within the inversion loops all contribute to bridge frequencies in inversion heterozygotes, according to Sarbhoy (29). In our material precise information on the location and number of inverted segments cannot be obtained because P. vulgaris and P. coccineus lack chromosome and linkage maps. The technique for detecting the location of inverted segments used by Morgan (26) cannot be applied at the present stage.

The quantitative relationship between bridge frequency and pollen abortion ratio was presented by Brown (3) and by Rhoades and Dempsey (28), using maize (Zea mays) as an example. In maize, when one bridge is present at anaphase I, two of the four pollen grains from that

PMC are expected to abort because of chromatid deficiencies resulting from a crossover within the inversion loop. The other two chromatids remain intact. They join the polar groups of chromosomes together with the broken chromatids when the bridge is broken. Thus, in maize the expected percentage of pollen abortion due to anaphase I events is half of the bridge percentage observed in anaphase I. For example, if 10% of the PMCs are observed to have a bridge at this stage, they are responsible for 5% pollen abortion in the pollen population of that plant.

The above calculation is based on two assumptions. The first is that the chromosome bridge breaks at anaphase I. The second is that the two bridged chromosomes join the polar groups after bridge breakage. The present study indicated that neither of these two assumptions is true in bean PMCs to the extent observed in maize. In the hundreds of PMCs studied it was noted that most of the bridges did not break at anaphase I. After telophase I, interphase, and prophase II, the bridges are still intact in metaphase II cells (Fig. 61-F). Furthermore, the bridge frequencies observed in clusters of metaphase II PMCs were always similar to those observed in anaphase I PMCs. Unlike the PMCs in maize, bean PMCs do not form a cellular wall to separate the daughter cells at telophase I. The formation of a cellular wall is responsible for cutting the chromosome bridge in maize at telophase I, but this does not happen in bean PMCs. A few bridges do break as a result of stretching in anaphase I. Consequently, the four chromatids of the bridged chromosomes become laggards in the middle metaphase II cells (Fig. 61-F). From the above observations we infer that none of the four chromatids will join the polar group when there is bridge

formation in the bean species studied. Furthermore, we conclude that in beans, the percentage of chromosome bridges in anaphase I is directly responsible for an equivalent percentage of abortion among pollen grains. In our material, chromosome bridges are responsible for 15.6, 16.8, and 25.6% pollen abortion in the three interspecific hybrids studied.

Since a small percentage of chromosome bridges break at anaphase I, the bridge frequency in metaphase II is always slightly lower than that of anaphase I. Since only the anaphase I bridge frequency is directly related to the pollen abortion ratio, it is necessary for the observer to distinguish the chromosome configurations of anaphase I and metaphase II in bean species. Any failure to distinguish between these two meiotic phases will underestimate the bridge frequency and pollen abortion ratio. Since the existence of chromosome bridges at the metaphase II stage has important implications in the cytogenetic analysis of bean, three major criteria are suggested here for discriminating between anaphase I cells and metaphase II cells (Fig. 61-B,C,E, and G).

1. In anaphase I, the chromosomes cluster around two points (poles), forming two spherical masses. In metaphase II, the chromosomes spread on two different planes (equatorial plates).

2. The chromosomes of anaphase I are dragged by spindle fibers. Although the tiny bean chromosomes do not show V or J shapes, they become folded and thicker as force is applied. Chromosomes at the metaphase II stage have just passed prophase II. Metaphase II chromosomes are relaxed and are not deformed by any dragging force. Therefore, metaphase II chromosomes have a sharp chromosome edge, in contrast to the folded and fuzzy-edged chromosomes of anaphase I.

3. Anaphase I chromosomes tend to uncoil or "melt" into interkinesis. Many proteinaceous ligands begin to connect the chromosomes at each pole. The metaphase II chromosomes tend to split into two chromatids and no ligand is formed.

Anaphase II Analysis

The meiotic aberrations observed as chromosome bridges and laggards in anaphase II (Table 3 and Fig. 61-G and H) are identical to those observed in anaphase I of the hybrids, La Vega x Pc-37 and 6-19 x Pc-50 (Table 2). They are 16.0 and 25.7%, respectively. The equal frequencies of bridge-laggards in these two phases were previously reported by Xolocotzi et al. (34). The mechanics of anaphase II bridge formation was discussed by McClintock (21) and by Brown (3). The present data confirm the equal bridge-laggards frequencies of anaphase I and anaphase II PMCs.

According to Brown (3), there are five types of pachytene configurations that give rise to anaphase I bridges: a single exchange or a three-strand double crossover within the inversion loop, a two- or four-strand double crossover with one crossover in the inversion loop and another in the proximal region, a three-strand double crossover with one in the inversion loop and another in the distal region, and a four-strand double crossover in the inversion loop. Only one pachytene configuration gives rise to anaphase II bridges. That is the three-strand double crossover with one in the inversion loop and another in the proximal region.

In this work, anaphase II bridge-laggard frequencies were observed as 16.0 and 25.7% in two of the hybrids. Identical bridge-

Table 3. Anaphase II bridge-laggard frequencies of P. vulgaris, P. coccineus and their hybrids.

<u>Species</u>	<u>Total cells</u>	<u>1 laggard</u>	<u>1 bridge</u>	<u>% abnormal</u>
6-19 (<i>vulgaris</i>)	45	0	0	0.0
L.V. (<i>vulgaris</i>)	40	0	0	0.0
Pc37 (<i>coccineus</i>)	44	0	0	0.0
F ₁ 6-19 x Pc37	63	4	2	9.5
F ₁ L.V. x Pc37	131	13	8	16.0
F ₁ 6-19 x Pc50	66	7	10	25.7

laggard frequencies were observed for anaphase I PMCs. Our difficulty is that we cannot explain the high frequencies of bridge-laggard events observed in anaphase II which required a particular type of double crossover event involving three strands for its occurrence.

It seems probable that the frequency of the four pachytene crossover events leading to anaphase I bridges (3) is greater than the frequency of three-strand double crossovers which produce bridges in anaphase II. In maize the expectation that anaphase I bridges should be higher than anaphase II bridges has been confirmed by experimental observations (21,28). However, our observations and those of Xolocotzi et al. (34) show that the bridge frequencies of anaphase I and II in bean are equal. The lower bridge-laggard frequency observed in anaphase II PMCs of the hybrid 6-19 x Pc-37 is probably caused by sampling error.

In bean plants, cytogenetic analysis cannot be done as precisely as in maize (21) because of the difficulty in detecting tiny acentric fragments and double bridges involving two chromatids of the same chromosome. Bean chromosomes are too small for these events to be observable. Also, bean chromosomes cannot be distinguished in pachytene phase. Thus, we have no way to determine the frequency of the various types of crossover events leading to bridges and fragments when an inversion is present.

According to the literature (3,28), the PMCs of maize with an anaphase II bridge are responsible for 50% of pollen sterility in pollen

derived from those PMCs. Thus, 8.0 and 12.8% pollen sterility (1/2 x 16% and 25.7%) in the bean hybrids La Vega x Pc-37 and 6-19 x Pc-50, respectively, are the direct result of anaphase II bridge-laggards events.

Unequal Distribution of Chromosomes at Anaphase I

It is difficult to find a sufficient number of PMCs with chromosomes well spread (without overlapping or connection) for chromosome distribution analysis. In such analysis, metaphase II cells are more suitable than anaphase I cells. Only 10 PMCs in this category were observed. Two of these PMCs showed 10 chromosomes at one pole and 12 chromosomes at the other (Fig. 6I-I). The sample was too small to provide a reliable estimate of the frequency of this aberration. However, it is certain that some fraction of the sterile pollen is due to unequal distribution of the chromosomes at anaphase I stage. The cause of this irregularity could be either asynapsis or both centromeres being dragged to one pole at anaphase I. Perhaps due to "spindle fiber failure" at one pole, both centromeres of the bridged chromosome pair were dragged entirely to one pole intact. In fact, one of the two PMCs in this analysis showed two chromosomes firmly connected among the group of 12 chromosomes.

A 10:12 distribution resulting from asynapsis is less likely because the frequency of two univalents observed in diakinesis was too low. It is more likely that this 10:12 distribution was produced by the breakage or stretching of one of the spindle fibers attached to a bridged chromosome in view of such high frequencies of chromosome bridges in

anaphase I. In this case, dragging the bridged chromosome to one pole may cause at least 50% pollen abortion in the tetrad, depending upon the behavior of the connected chromosomes during anaphase II. It may form a chromosome bridge if the connected chromatids are pulled to opposite poles.

Normal Pollen Abortion

In normal plants pollen abortion can occur as high as 5% (26). We observed 2% in P. coccineus and less than 1% in P. vulgaris. This fraction should be considered in the makeup of pollen sterility observed in those interspecific hybrids studied.

F₂ Seed Development

The mature F₂ seeds harvested from plants of the Great Northern #1 sel. 27 x Pc-37 hybrid showed a wide distribution in terms of seed development (Fig. 61-J). Only 30% of the 155 seeds were fully developed. Another 30% of the seeds were shrunken, with each individual seed weight in this group less than 200 mg. The remaining 40% of the seeds were intermediate in development. The present data on F₁ meiotic analysis do not show any direct relationship between chromosome aberrations and the causes of underdevelopment of F₂ seeds.

Aneuploid Progeny in Interspecific F₂

The observation of metaphase II cells with bridge-laggard and with unequal distribution of chromosomes at two poles raises the possibility that aneuploid progeny may be produced by chromosome inversions in bean species if the abnormal megaspores can survive. Since at least two inversions in different pairs of chromosomes are present in these two species, many types of aneuploids can be obtained, including monosomics and trisomics.

CONCLUSIONS

The above analytical data indicate conclusively that the genomes of P. vulgaris and P. coccineus cannot be considered as identical in chromosome structure. In the materials studied, at least two chromosome inversions located in different pairs of chromosomes and associated with high crossing over rates account for nearly half of the pollen abortion observed in the interspecific hybrids. If we consider additional percentages of pollen abortion due to unequal distribution of chromosomes at anaphase I and the occurrence of normal pollen abortion, the total fraction of pollen abortion caused by gene unbalance may be less than half of the observed percentages. These data also help to explain the results obtained by Smartt and Haq (30) in which the pollen fertility was improved from 21% in the interspecific hybrids to 42% in the amphidiploids, and reaching 76% by further selection with inbreeding. The residual 25% of pollen sterility was probably due to homozygous genic imbalances which could not be removed by selection.

PART III

CHROMOSOME IDENTIFICATION AT THE DIPLTENE STAGE OF
MEIOSIS IN SCARLET RUNNER BEAN (Phaseolus coccineus)

INTRODUCTION

The identification of each chromosome of the genome of a plant species is a very important step in the development of cytogenetic knowledge about that species. Once individual chromosomes can be identified on the basis of their morphology, it becomes much easier and requires less time to perform additional cytogenetic studies, e.g., the association of genes with a particular chromosome, the detection of structural differentiation within or between species, and developing aneuploid and translocation stocks.

In maize, different knob positions and centromere location on each of the 10 chromosomes were used to identify each chromosome (20,27). In the tomato plant, centromeric constitutive heterochromatin zones are present in every one of the 12 chromosomes. The banding patterns of such centromeric zones, the ratio between the lengths of the two arms of the chromosomes, and the length of distal euchromatin are used for tomato chromosome identification (1,22). The chromosome map and linkage map for maize and tomato have been worked out thoroughly, and extensive practical application of this knowledge has been made.

Beans (Phaseolus vulgaris and Phaseolus coccineus) are important field crops throughout the world. However, their chromosome maps have not been established. Because of the unavailability of a chromosome map, a complete genetic linkage map would be difficult to construct through the use of aneuploid stocks.

A bean chromosome map has not been developed previously because the metaphase chromosomes are too small to be identified and the pachytene chromosomes are difficult to stain. Pachytene chromosomes of these species are very long and overlap each other extensively in a flattened PMC. Thus under conventional staining technique, their morphology cannot be studied. The Giemsa banding pattern of mitotic prophase chromosomes proposed by Mok and Mok (24) is difficult to use because the patterns are so similar.

Several attempts to establish chromosome morphology in Phaseolus aureus, Phaseolus mungo and tetraploid Phaseolus species in pachytene and mitotic reproductive phases do not correlate with each other (14). The chromosome condensation from the pachytene stage to metaphase in tomato was analyzed carefully by Brown in 1949 (2). He observed that the heterochromatic zones of pachytene chromosomes condense much less than euchromatic zones during chromosome condensation and that euchromatic zones contribute very little to the chromosome length at metaphase. These observations are still in close agreement with the up-to-date model of chromosome structure presented by Comings (5). The discrepancies between mitotic and meiotic chromosome maps in P. aureus cannot be corrected using Brown's condensation principles (2). The trouble lies in the technical difficulties of studying bean pachytene and metaphase stages. The photomicrographs of P. aureus pachytene cells do not show distinct centromeric heterochromatin zones similar to those found in tomato pachytene (1). On the contrary, the chromatic bodies are well distributed along the chromosome arms which are neglected as euchromatin. Therefore, the metaphase map showed too much heterochromatin complement as compared with the pachytene map.

Besides the difficulties described above, a quantitative error in pachytene measurement is produced by camera lucida drawings which neglect the tilting and folding-under effects. The pachytene chromosomes are so entangled that it is difficult to obtain a precise measurement of chromosome length.

The metaphase chromosomes of Phaseolus are very small. Slight tilting can give rise to considerable measurement error. In this stage no banding pattern can be observed for chromosome identification.

The above considerations led to the idea of chromosome identification in the diplotene stage in beans. Because the chromosomes in this stage are shorter than those of pachytene stage, most of them are free from overlapping. They also have characteristic banding patterns for identification. The two chromosomes are paired but not synapsed. The presence of two slightly separated homologues increases the contrast and reliability of the banding patterns.

In this work a new staining procedure was developed to study the morphology and banding patterns of the diplotene stage chromosomes of Phaseolus coccineus, which is a closely related species of the common bean (Phaseolus vulgaris).

LITERATURE REVIEW

The subject of chromosome structure and banding patterns has been reviewed by Comings (5). According to his paper, there are three kinds of banding structures in a chromosome:

1. Centromeric heterochromatin is a dark staining zone located on both sides of the centromeres. In tomato pachytene, this structure is seen in all 12 chromosomes (1,22).
2. Intercalary heterochromatin is distributed along the chromosome arms. These segments are observed as distinct dark staining bodies in pachytene chromosomes.
3. Euchromatin is weakly stained or non-stainable zones in the chromosome arms.

Comings also presented the model of chromatin packing in mammalian chromosomes based on current knowledge from DNA research. The model describes the structure of small and large chromomeres, banding bodies, chromosome spiralization, and condensed chromosome morphology.

According to this author, the traditional cytogenetic stains generally give G band patterns on the chromosomes. In the staining process, the alcohol and acetic acid fixation of dividing cells removes protein molecules attached to the chromosome and increases the binding of dye to the chromosome. The distinct chromatic zones observed in prophase chromosomes are directly correlated to the tightly packed heterochromatin bodies which contain large amounts of DNA and nucleoprotein

to bind large quantities of dye. Euchromatic zones are composed of loosely packed chromatin containing a small amount of genetic material.

Barton (1) established definitively the pachytene morphology of the tomato genome by camera lucida drawings. He identified each chromosome by total length, short arm length, long arm length, arm ratio and the chromatic and achromatic regions on both short and long arms. Since camera lucida drawings record a three dimensional object (a cell in pachytene stage) as a two dimensional diagram, the length of the chromatin involved in a third dimension (depth) is either reduced or lost completely. Therefore, pachytene chromosome maps made by different observers always present discrepancies. However, the centromeric heterochromatins of tomato pachytene chromosomes are so distinct that there is no difficulty in identifying the different chromosomes.

Menzel (22) failed to detect any structural difference in pachytene between Lycopersicon esculentum and Solanum lycopersicoides by comparing pachytene banding patterns of parental and interspecific F_1 hybrid plants. Nevertheless, the amphidiploid data showed that a great portion of pollen sterility in the interspecific F_1 was due to chromosome structural differences. This paper clearly demonstrates the difficulty in interpreting pachytene configurations. In spite of the presence of many pachytene loops, she could not distinguish whether the loops were the result of an inversion, an addition, a deficiency, or just asynapsis. Two pachytene segments with the same length can appear totally unequal when they are arranged in loops with tilt orientation. Thus, despite the fact that pachytene analysis is useful for chromosome identification in tomato and other species, its precision in detecting

structural differences is inferior to analysis of anaphase and other meiotic stages.

Krishnan and De (7,14,15) tried to establish the morphology of individual chromosomes of Phaseolus species at pachytene and metaphase stages in order to study chromosome differentiation among the species of this genus. Unfortunately, the pachytene configurations found in P. aureus do not have distinct centromeric heterochromatin regions for identification. The chromatic bands in P. aureus pachytenes are more or less distributed along the chromosomes. The centromeric regions do not have a distinctive morphology like those in tomato pachytene. Therefore, the chromosome model built according to pachytene observations does not correspond with the model derived from metaphase cells. In one paper, they were unable to identify four of the chromosomes (7). However, their pachytene studies led them to conclude there were chromosome aberrations such as translocations, duplications, and deficiencies distinguishing P. aureus and P. mungo.

The Giemsa banding model of bean prophase chromosomes proposed by Mok and Mok (24) gave less detail and led to more ambiguity than pachytene morphology. The chromosomes are too short and the banding too broad. The short chromosomes simply provide too few differentiating regions for identification. There is no possibility to study structural differences between bean genomes with this banding technique.

Brown (2) studied the condensation process of tomato chromosome #2, the nucleolar chromosome, from pachytene to metaphase. He concluded that the heterochromatin zones at both sides of centromere at pachytene contribute to most of the chromosome length at metaphase. The longer

euchromatic distal regions make a negligible contribution to chromosome length at this stage. He attributed all the condensation to euchromatin packing, while the heterochromatin bodies do not contract at all. This hypothesis is in close agreement with Comings' model of chromosome packing (5).

MATERIAL AND METHODS

The bud samples from scarlet runner beans (*P. coccineus*) used in this study were collected at the beginning of the flowering period. The flower buds were harvested at 9:30 a.m. for several days in May 1979. The buds were immersed immediately in Carnoy's fixative solution containing 6:3:1 of ethanol(95%):chloroform:glacial acetic acid by volume and treated for 24 hours. They were transferred to 70% alcohol for preservation.

Anthers were extracted from selected flower buds which ranged in length from 2.5 to 3.0 mm, using a stereo dissecting microscope. About 50 anthers were put in the well of a porcelain dish for staining. Three drops (0.5 ml) of 45% iron propionic carmine were added to stain the PMCs within the anthers for 1.0 to 1.5 hours. During this period, the stain dries to 1/3 of the original volume. The anthers were then transferred to a slide for squashing, using an ultrafine dissecting tweezer (A. Dumont & Fils #5). Temporary slides were sealed with Kerr Sticky Wax. The slides were good for one week. After that period, the cells began to shrink and debris accumulated around the cell surface. Therefore, all the photomicrographs were taken during the first 3 days after slide preparation, using a phase contrast system under 1000 X magnification. High contrast black and white films, Panatomic X with ASA 32, and color slide film, Kodachrome with ASA 25, were used for photomicrographs. The slides were searched for diplotene cells having

chromosomes with favorable orientation. The morphology of the 11 chromosomes was studied from at least five photographs of the same chromosome in the most favorable orientation in five different diplotene cells. When several pairs of diplotene chromosomes were present in favorable orientation in one cell, their relative lengths were measured in order to arrange the 11 chromosomes in approximate rank order from longest to shortest. For measuring the relative lengths of the chromosomes, the color slides were projected to a screen 15 feet from the projector. The apparent length of each chromosome was measured, and from these data the relative lengths of the 11 chromosomes were calculated. Finally, an idiogram was built according to the relative length and specific banding patterns of each chromosome. Numbers were assigned to the 11 chromosomes in the rank order, 1 being the longest and 11 being the shortest.

RESULTS AND DISCUSSION

Overlapped and entangled chromosomes in the prophase stage have been the major obstacle in chromosome identification in bean and other species. The problem was partially solved by searching for large PMCs which provide more space for chromosome distribution, reducing overlapping and entanglement. From searching many meiotic slides for PMCs, it was learned that there are considerable differences in the size of PMCs within a plant. The large PMCs can be twice as big as small PMCs. The large PMCs are produced almost exclusively at the beginning of flowering. The PMCs collected during the middle or late parts of the flowering period were usually small. Therefore, the materials for prophase chromosome identification were all harvested before and during anthesis as early in the flowering stage as possible.

Another problem encountered in prophase chromosome identification is the difficulty in obtaining a high contrast between the chromosome and the cytoplasmic background. It was discovered that the fixation process has important effects on the contrast problem. In order to have lightly stained cytoplasm after staining, the flower buds should be thoroughly bleached during the fixation process by using an excessive quantity of fixative. When only a small amount of fixative is used, the fixative solution becomes very dark with the breakdown of chlorophyll from the bud clusters. The dark solution tends to darken the cytoplasm and reduces the contrast between the cytoplasm and chromosomes.

In order to obtain PMCs with clear cytoplasm, at least 100 ml of fixative should be used for the fixation of 50 bud clusters.

Besides the cell size and fixation problems, the conventional chromosome staining procedure was found to be inadequate for staining prophase chromosomes in bean for identification. The standard staining solution with 45% iron propionic carmine was adequate for staining chromosomes at diakinesis, metaphase and anaphase. Unfortunately, it did not stain prophase chromosomes satisfactorily. To find out if the inadequate staining of prophase chromosomes was due to stain concentration, an experiment was performed, using three treatments:

1. Constant concentration of 45% iron propionic carmine in which the anthers were immersed in a porcelain well sealed with a cover-glass and wet cheese cloth for 2 hours.

2. Small concentration gradient in which the excessive stain (1.5 ml) was put in a porcelain well with anthers for 1.5 hours without covering the well.

3. A steep concentration gradient in which 3 drops (0.5 ml) of the stain were put in the well with anthers for 1.5 hours without covering the well.

After squashing anthers from each treatment and mounting the slides, the darkly stained prophase chromosomes with high contrast were found only in the third treatment. The leptotene, pachytene and diplotene chromosomes with their characteristic dark banding patterns could be seen distinctly against the pale pink cytoplasm. The PMCs from the first two treatments did not show clear chromosome threads. Therefore, it was concluded that a steep concentration gradient is necessary for

the carmine to stain prophase chromosomes from leptotene to diplotene stage. The technique was adopted to study the diplotene morphology in this work and to complete the photomicrographic illustration of the meiotic sequence in Part I. This new staining technique is adequate for staining meiotic chromosomes at all the stages from interphase to pollen mitosis. Therefore, it increases the number of observable PMCs in each slide.

The banding patterns in diplotene are very specific for each chromosome. Using the new staining procedure, the chromosomes show dark heterochromatic bands and clear euchromatic zones. The heterochromatic zones are different in thickness along the chromosome arms. Therefore, by the different combination of thickness and band length, it is possible to distinguish one chromosome from another. The relative length is useful to separate the long chromosomes from the short chromosomes, although this character is not sufficiently accurate for identifying chromosomes of similar length. It is fortunate that no two diplotene chromosomes possess similar banding patterns, which might confound the identification work. The individual chromosome morphology and its banding pattern were unique for each chromosome.

For each chromosome (Figs. 62 through 72) there is presented a series of photomicrographs, A, B, C, etc., showing only that chromosome. Each of the lettered photomicrographs has a drawing directly below it. These photos were cut out of a print originally including an entire diplotene PMC. At the bottom of each figure (Figs. 62 through 72) there is an idiogram for that chromosome. None of the idiograms has a centromere because it was impossible to locate the centromere position

in any of the chromosomes in the diplotene PMCs studied. The figures are presented in the order of chromosome length, #1 being the longest. Chromosomes #2 through #6 are indistinguishable in length but are shorter than #1. Chromosomes #7 and #8 are similar in length but are shorter than #2 through #6. Chromosomes #9, #10, and #11 are each shorter than the previous ones, and as a group they are distinctly shorter than chromosomes #1 through #8.

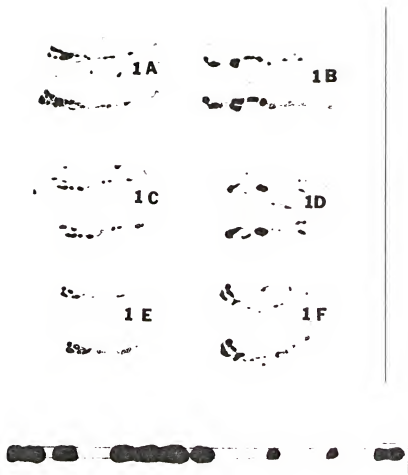


Figure 62. Chromosome #1 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #1 (Fig. 62) is the longest diplotene chromosome in most of the PMCs. It is easily identified by its long achromatic arm extending about half of the chromosome length with two lightly stained bodies separated by equal distances and a dark telomere. The other half is darkly banded. In this region three major bands are present. The longest band is located near the achromatic half, the second band in the terminal region, and a minor band between the two longer bands. Thus, this chromosome is half dark and half achromatic with a dark telomere at the achromatic end.

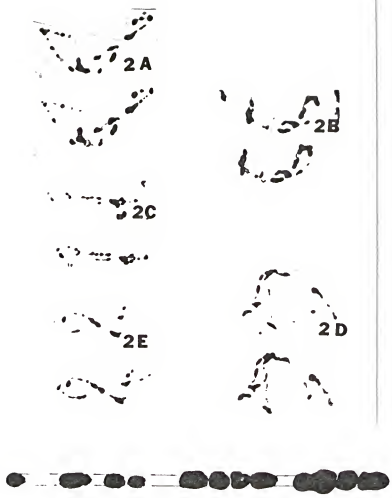


Figure 63. Chromosome #2 of *Phaseolus coccineus* in the diplotene stage of meiosis.

Chromosome #2 (Fig. 63) is as long as chromosome #1. It has three bands nearly equal in length, two of them at the ends and one in the middle region of the chromosome. Each band occupies slightly less than 1/3 of the chromosome. They are all composed of four dark-staining bodies (Fig. 63-C). The dark-staining bodies are very dark in the band at one end, less dark in the middle band, and light in the band at the other end. Two achromatic zones separated the three bands.

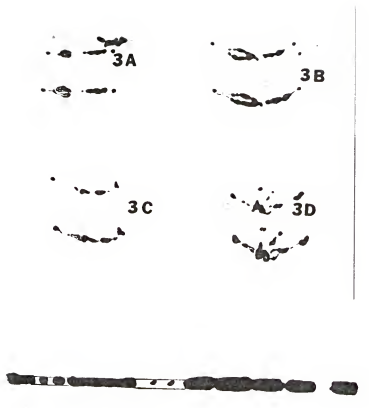


Figure 64. Chromosome #3 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #3 (Fig. 64) has a dark telomere at each end, one is more distinct than the other. An achromatic zone located in the middle region occupies nearly 1/5 of chromosome length in which two lightly stained bodies sometimes appear. Near the darker telomere, a band is constituted of 3 dark chromatic bodies and a light chromatic body. The other band near the lighter telomere is about 1/3 longer than the previous band and is composed of four dark chromatic bodies and two lighter chromatic bodies.

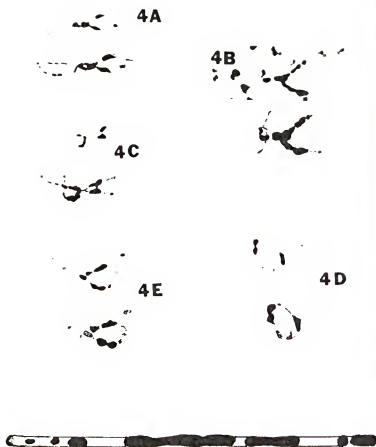


Figure 65. Chromosome #4 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #4 (Fig. 65) has two dark bands located in the middle region of the chromosome. The band located at the center of the chromosome is longer than the submedian band. Near this shorter band, there is an achromatic region as long as this band. A dark telomere appears at the end of this achromatic zone. At the other arm beyond the long band, it is all achromatic with three to four lightly stained chromatic bodies spread along the arm. One of these bodies is a telomere.

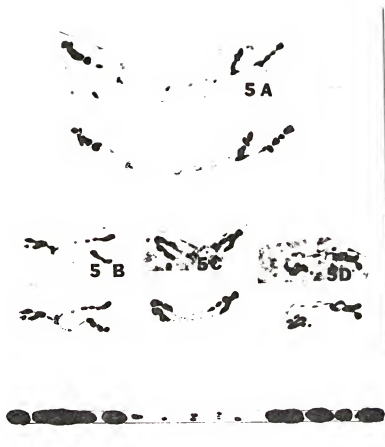


Figure 66. Chromosome #5 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #5 (Fig. 66) has a very long achromatic region in the middle of the chromosome occupying 3/5 of the chromosome length. Two dark bands, one longer than the other, are located at both ends. This is a chromosome contrary to the theory of centromeric heterochromatin reported by Krishnan and De (15) in Phaseolus aureus and by Barton (1) in tomato. In this chromosome, the whole central region, where the centromere is supposed to be located, is all achromatic, with 5 to 6 inconspicuous bodies distributed along this region. This chromosome is frequently misinterpreted as two separate chromosomes, sometimes as late as the early diakinesis stage.

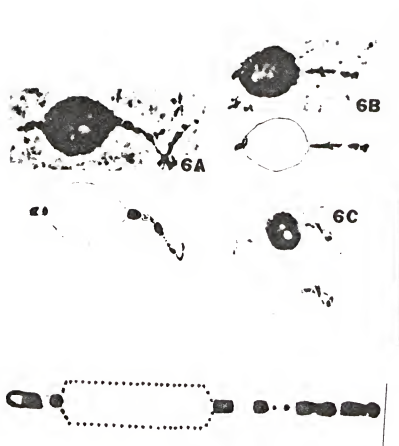


Figure 67. Chromosome #6 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #6 (Fig. 67) is also as long as the previous chromosomes, #2 through #5. It contains the nucleolar organizer near one end. At the opposite end, a very dark telomere is present. Near this telomere there is a long dark band. Two smaller dark bands are located in the long arm near the nucleolus. The short arm does not have a dark telomere. Only two small bands are located near this achromatic telomere. This nucleolar chromosome is the easiest among the 11 chromosomes to be identified. Its morphology is also the most difficult to observe because of the nucleolar mass.



Figure 68. Chromosome #7 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #7 (Fig. 68) is apparently shorter than the previous chromosomes. The chromatic bands are all located in one arm occupying less than half of the chromosome. The banding region is composed of three continuous chromatic lobes, and the telomere is a lighter lobe. More than half of this chromosome is achromatic with a lightly stained telomere. A few inconspicuous bodies are distributed along the achromatic region.

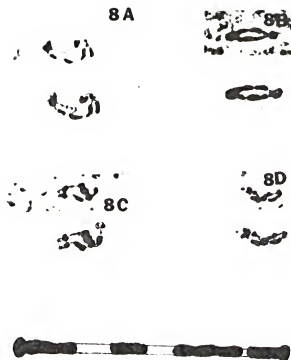


Figure 69. Chromosome #8 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #8 (Fig. 69) is as long as chromosome #7. It contains four segments of dark banding, two at the ends and two around the center. These two bands are different in length. The three achromatic segments that separate the banding regions into four sections are sometimes not very distinct (Fig. 69-B). The two telomeres, which are part of the terminal bands, are very distinct.

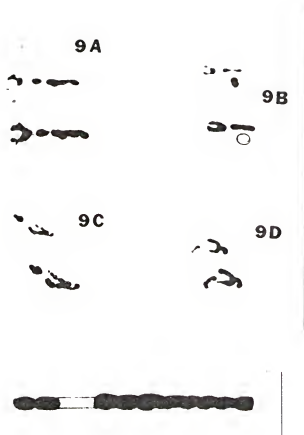


Figure 70. Chromosome #9 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #9 (Fig. 70) has one band at each end. The longer band occupies about 7/10 of the chromosome and the shorter band occupies 2/10. The achromatic region that separates the two bands is about 1/10 of the chromosome length. The long band is composed of at least four chromatic lobes. The one near the achromatic zone is sometimes separated from the other three lobes (Fig. 70-A).

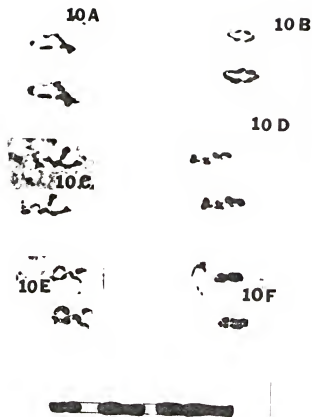


Figure 71. Chromosome #10 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #10 (Fig. 71) is a short chromosome containing three chromatic bands separated by two achromatic zones. The three bands are different in length. The longest band is located at one end and the shortest band at the other end. The middle band is intermediate in length. The approximate length ratio of these three bands is 3:2:1.

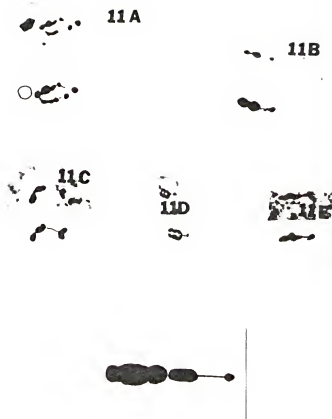


Figure 72. Chromosome #11 of Phaseolus coccineus in the diplotene stage of meiosis.

Chromosome #11 (Fig. 72) has four dark chromatic bodies at one end which are sometimes connected into two similar lobes. The band region occupies about half of the chromosome. The other half of the chromosome is composed of a thin achromatic thread with a distinct telomere at the end. A small nucleolar-like body is frequently associated with the chromatic end of this chromosome.

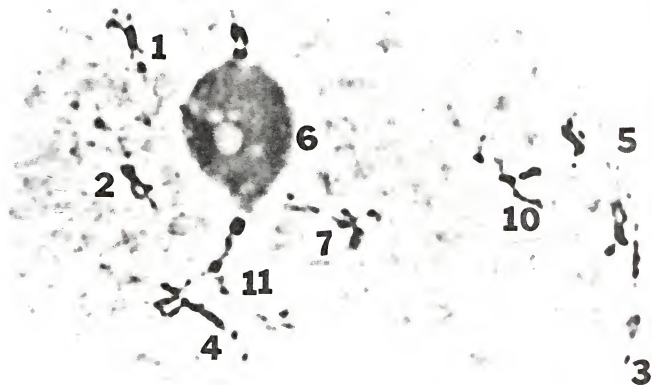


Figure 73. A Phaseolus coccineus PMC at the diplotene stage of meiosis.

Nine chromosomes are identified by number. Chromosomes #8 and #9 are not identifiable in this PMC.

Attempts to match the present diplotene model with Mok and Mok's somatic prophase model (24) failed because of a lack of structural similarity. Therefore, a new numeric order similar to that used in maize and tomato was adopted for this model. Among the 11 chromosomes of Mok and Mok's model, four of them have an overall banding pattern similar to the present model although their relative lengths are different. They are: chromosome F, similar to chromosome #9; chromosome H, similar to chromosome #5; chromosome J, similar to chromosome #11; and chromosome K, similar to chromosome #4. In order to differentiate all the chromosomes from all others, a stage with abundant morphological detail is necessary. Mok and Mok's model is ambiguous because few differentiated regions are evident in somatic prophase.

The morphology of the 11 chromosomes of Phaseolus coccineus presented above is useful for chromosome identification in translocation and aneuploid studies. It may not be an effective tool for detecting chromosome structural aberrations unless the segment involved in the aberration is very large and includes different banding regions.

CONCLUSION

The new staining procedure with iron propionic carmine shows that diplotene chromosomes of Phaseolus coccineus have unique banding patterns which can be used for identification of each chromosome. Their banding patterns are presented in idiograms and photomicrographs in this paper. Their potential for application in other cytogenetic studies is discussed.

SUMMARY

The new staining procedure with 45% iron propionic carmine shows that diplotene chromosomes of Phaseolus coccineus have unique morphology and banding patterns. The relative length of the chromosomes is also useful for identification of each chromosome. The banding patterns and relative lengths are presented in an idiogram and photomicrographs in this paper. The chromosome models presented in this work will probably be most useful in identifying which chromosomes are involved in trisomic and translocation heterozygotes, either directly or by elimination of the chromosomes not involved in the anomalous configuration.

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BIOGRAPHICAL SKETCH

Simon Suhwen Cheng was born February 4, 1944, in Tai-Chung, Taiwan, Republic of China. He graduated from the First Provincial High School in that city in 1962, and in 1967 he received a B.S. degree in agronomy from the National Taiwan University, Taipei, Taiwan. He emigrated to Brazil in 1968 and received an M.S. degree in horticulture from the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, in 1972. Currently, he is an Assistant Professor in the Department of Agriculture in Escola Superior de Agricultura de Lavras, Lavras, Minas Gerais, Brazil.

He married Elizabeth Ying Chu who gave him three children: Henrique, Isabela and Spencer.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Mark J. Bassett
Mark J. Bassett, Chairman
Associate Professor of Vegetable Crops

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Chesley B. Hall
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Professor of Vegetable Crops

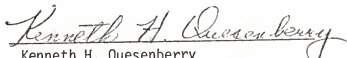
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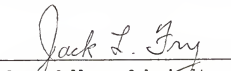
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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1979


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